Hemoglobin Assay Kit

Catalog # 6024

For Research Use Only - Not Human or Therapeutic Use

PRODUCT SPECIFICATIONS

DESCRIPTION:	Assay kit to quantify hemoglobin
FORMAT:	96-well ELISA Plate with removeable strips
ASSAY TYPE:	Colorimetric Assay
ASSAY TIME:	15 minutes
STANDARD RANGE:	2 mg/ml to 0.03 mg/ml
NUMBER OF SAMPLES:	Up to 40 (duplicate) samples/plate
SAMPLE TYPES:	Blood, Serum, and Plasma
RECOMMENDED SAMPLE DILUTIONS:	1:200 (Blood)
CHROMOGEN:	N/A (read at 400 nm)
STORAGE:	4°C
VALIDATION DATA:	Intra-Assay (5.7-6.7%)/Inter-Assay (2.7-4.5%)/Spiking Test (103-106%)
NOTES:	

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INTRODUCTION

Hemoglobin is a heme protein carried by erythrocytes and transports oxygen from the lungs to the rest of the body. Therefore, low hemoglobin levels in erythrocytes or low erythrocyte numbers in blood deprives the body of oxygen. This condition is defined as anemia. This oxygen deficit can cause dizziness or headaches, while severe or long-lasting anemia can damage organs in the body and even result in death. Anemia is also a complication of various inflammatory diseases such as sepsis (1-3) and autoimmune diseases (4, 5), and has been observed in rodent disease models of inflammatory bowel disease (IBD) (5, 6), colitis (7), sepsis (8), and autoimmune hemolytic anemia (9-12).

As hemoglobin levels correlate to the oxygen carrying-capacity of blood, hemoglobin is a useful marker of anemia to evaluate disease severity and effectiveness of treatments. A standard hemoglobin assay utilizes the toxic cyanmethemoglobin method. However, Chondrex, Inc. would like to introduce a safe, non-cyanide hemoglobin assay kit (13, 14). This assay kit correlates well with the cyanmethemoglobin method and can be used to evaluate anemia in our mouse sepsis model and mouse IBD model, as well as other disease models. Chondrex, Inc. also provides related products such as FITC-Dextran, mouse anti-bacterial antibody ELISAs, and an HMGB1 Detection ELISA kit. For more information, please visit www.chondrex.com or contact support@chondrex.com.

KIT COMPONENTS

Item	Quantity	Amount	Storage
Bovine Hemoglobin Standard (60241)	1 vial	Lyophilized, 2 mg	4°C
Reaction Solution (60242)	1 bottle	20 ml	4°C
ELISA Plate	1 plate	96-well (8-well strips x 12)	4°C

ASSAY OUTLINE

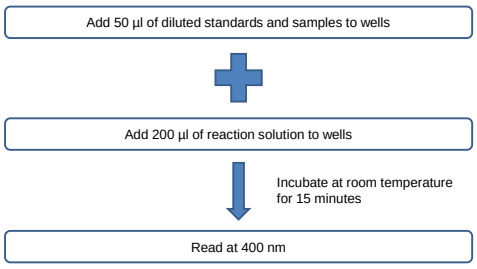

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PLATE LAYOUT

	1	2	3	4	5	6	7	8	9	10	11	12
A	2	2	S1	S1	S9	S9	S17	S17	S25	S25	S33	S33
B	1	1	S2	S2	S10	S10	S18	S18	S26	S26	S34	S34
C	0.5	0.5	S3	S3	S11	S11	S19	S19	S27	S27	S35	S35
D	0.25	0.25	S4	S4	S12	S12	S20	S20	S28	S28	S36	S36
E	0.125	0.125	S5	S5	S13	S13	S21	S21	S29	S29	S37	S37
F	0.63	0.63	S6	S6	S14	S14	S22	S22	S30	S30	S38	S38
G	0.31	0.31	S7	S7	S15	S15	S23	S23	S31	S31	S39	S39
H	B	B	S8	S8	S16	S16	S24	S24	S32	S32	S40	S40

Standards Samples

NOTES BEFORE USING ASSAY

NOTE 1: It is recommended that the standard and samples be run in duplicate.

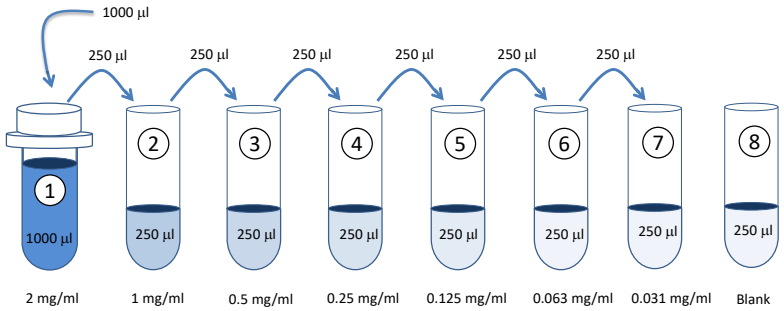
NOTE 2: Warm up all buffers to room temperature before use.

NOTE 3: Crystals may form in Wash Buffer, 20X when stored at cold temperatures. If crystals have formed, warm the wash buffer by placing the bottle in warm water until crystals are completely dissolved.

NOTE 4: Measure exact volume of buffers using a serological pipet, as extra buffer is provided.

ASSAY PROCEDURE

1. **Prepare Standard Dilutions**: Reconstitute one vial of Standard with 1 ml of distilled water to make a 2 mg/ml standard solution. Then serially dilute the 2 mg/ml solution with distilled water. For example, mix 250 µl of the 2 mg/ml solution with an equal volume of distilled water to make a 1 mg/ml solution, and then repeat it five more times to make 0.5, 0.25, 0.125, 0.063, and 0.031 mg/ml solutions. Chondrex, Inc. recommends making fresh serial dilutions for each assay.



2. **Prepare Sample dilutions**: Blood samples should be diluted at 1:200 in distilled water while serum and plasma samples can be assayed directly.

   NOTE: the sample dilution depends on the animal disease models.

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3. **Add Standards and Samples**: Add 50 µl of standards, distilled water (blank), and diluted samples to wells in duplicate. Pipet carefully to avoid bubbles in the wells.

4. **Add Reaction Solution**: Add 200 µl of Reaction Solution to each well and tap plate lightly to mix. Pipet carefully to avoid bubbles in the wells. Incubate for 15 minutes at room temperature.

5. **Read plate**: Read the plate at 400 nm (390-405 nm).

CALCULATING RESULTS

1. Average the duplicate OD values for the blank (and sample blank), standards, and test samples.

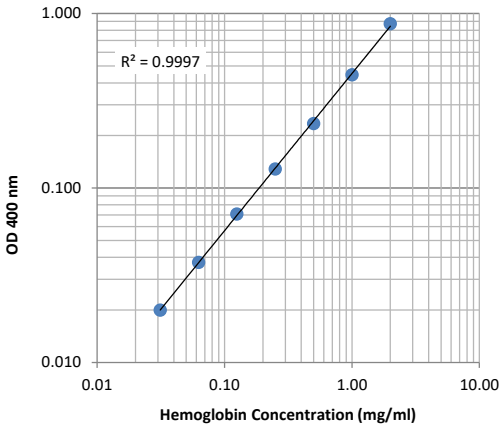
2. Subtract the "blank" (B) values from the averaged OD values of standards and test samples in Step 1

3. Plot the OD values of standards against the concentration of hemoglobin (mg/ml). Using a log/log plot will linearize the data. Figure 1 shows a representative experiment where the standard range is from 0.03 - 2 mg/ml.

4. The concentration (mg/ml) of hemoglobin in test samples can be calculated using regression analysis. Multiply the calculated hemoglobin levels by the sample dilution factor to obtain the hemoglobin concentration (mg/ml) in the original sample specimen. For additional assistance, please download a sample calculation worksheet from www.chondrex.com.

   NOTE: Conversions: 1 mg/ml hemoglobin equals 100 mg/dl hemoglobin, 15.6 µM, 0.1%, or 1000 ppm.

Figure 1 - A Typical Standard Curve for the Hemoglobin Assay Kit



VALIDATION DATA

Reproducibility Data for the Hemoglobin Assay Kit

Test At	0.06 mg/ml	0.3 mg/ml	1 mg/ml
Intra-Assay CV (%)	5.7	6.7	6.3
Inter-Assay CV (%)	2.7	4.2	4.5
Spiking Test*	106%	103%	105%

*Standard was added with known amounts of Hemoglobin and then diluted with distilled water for assaying hemoglobin.

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TROUBLESHOOTING

For frequently asked questions about assays and ELISAs, please see Chondrex, Inc.'s Assay FAQ for more information.

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